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Millions of Years Behind: Slow Adaptation of Ruminants to Grasslands

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Abstract.—The Late Cretaceous appearance of grasses, followed by the Cenozoic advancement of grasslands as dominant biomes, has contributed to the evolution of a range of specialized herbivores adapted to new diets, as well as to increasingly open and arid habitats. Many mammals including ruminants, the most diversified ungulate suborder, evolved high-crowned (hypsodont) teeth as an adaptation to tooth-wearing diets and habitats. The impact of different causes of tooth wear is still a matter of debate, and the temporal pattern of hypsodonty evolution in relation to the evolution of grasslands remains unclear. We present an improved time-calibrated molecular phylogeny of Cetartiodactyla, with phylogenetic reconstruction of ancestral ruminant diets and habitats, based on characteristics of extant taxa. Using this timeline, as well as the fossil record of grasslands, we conduct phylogenetic comparative analyses showing that hypsodonty in ruminants evolved as an adaptation to both diet and habitat. Our results demonstrate a slow, perhaps constrained, evolution of hypsodonty toward estimated optimal states, excluding the possibility of immediate adaptation. This augments recent findings that slow adaptation is not uncommon on million-year time scales. [Macroevolution; hypsodonty; Cetartiodactyla; ruminant; grassland; phylogenetic comparative method; Ornstein–Uhlenbeck process; adaptation.]

Evolution of high-crowned (hypsodont) teeth in ungulates and other herbivores is a textbook example of adaptive evolution across macroevolutionary time scales (Osborn 1910; Simpson 1944; Van Valen 1960; Janis 1988; Janis and Fortelius 1988; MacFadden 1992; reviewed in Damuth and Janis 2011). Apart from adaptations in the feeding apparatus, evolution of herbivory was followed by morphological and physiological adaptations especially in relation to body size (Demment and Van Soest 1985). There is a consensus that independent evolution of hypsodonty in a number of ungulate orders is an adaptation to herbivorous, tooth-wearing diet in increasingly open and arid habitats (e.g., Simpson 1944, 1953; Stirton 1947; Webb 1977; Fortelius 1985; Janis and Fortelius 1988; Jernvall and Fortelius 2002; Strömberg 2006). Grasses first appeared in the fossil record during the Late Cretaceous, 100.5–66.0 Ma (million years [myr] ago), and rose to dominance during the Cenozoic (Strömberg 2011). Grasslands replaced forests on most continents, purportedly due to global climate changes, albeit following different temporal patterns (Jacobs et al. 1999; Edwards et al. 2010; Strömberg 2011). Against this backdrop of environmental change, global ungulate diversification was most likely a result of adaptation to new habitats and diets. Multiple lineages of ungulate ancestors that were previously browsers evolved high-crowned molars, presumably due to selection imposed by more abrasive food in their diets such as grasses containing silica, covered in airborne grit and dust (Fortelius 1985; Janis 1988; Janis and Fortelius 1988; Jardine et al. 2012). Hypsodont teeth are vertically elongated in comparison with ancestral low-crowned brachyodont teeth and last longer because there is more tooth material to wear (Janis 1988; Janis and Fortelius 1988; Damuth and Janis 2011). Hence, hypsodonty may

prolong lifespan (e.g., Kurtén 1953; Van Valen 1960, 1964; Gaillard et al. 2000; Pérez-Barbería et al. 2015). Tooth morphology reflects the feeding habits of animals (Butler 1983; Fortelius 1985), and hypsodonty has been used as a tool in paleontology to infer diets and environments of extinct taxa (Eronen et al. 2010b; see Damuth and Janis 2011 for an overview), although it has been suggested that it needs to be supplemented with other cranial and tooth measures to adequately predict diets (Janis 1988; Fortelius and Solounias 2000).

More recently, Damuth and Janis (2011) and Strömberg (2011) have argued that the evidence for this classic story of adaptation is neither clear nor straightforward. The impact of different causative agents of tooth wear is still a matter of debate, in particular as to the different contributions of diet and habitat to hypsodonty evolution (Damuth and Janis 2011; Jardine et al. 2012). Additionally, there is increasing interest in the timing of hypsodonty evolution in relation to the expansion of grasslands (Damuth and Janis 2011; Strömberg 2011). The global shift from forests to C₃ grasses and the expansion of the latter to become the Earth's dominant terrestrial biome was followed by the subsequent transition to grasses with C₄ photosynthesis and their rise to dominance, in which a notable lag has been observed (Edwards et al. 2010; Strömberg 2011). The fossil evidence for these biotic shifts shows different temporal patterns on different continents. Several studies examining the timing of diversification of hypsodont lineages have found a lag between the spread of open-habitat grasses and the emergence of fully hypsodont taxa, ranging from 3 to 12 myr (Hansen 1997; Muhlbachler and Solounias 2006; Strömberg 2006; Muhlbachler et al. 2011). Despite the evolution of hypsodonty being a classic example of adaptation causing evolutionary trends over long-time scales, only

a few studies have investigated this hypothesis in an explicit phylogenetic comparative framework (Hansen 1997; Williams and Kay 2001; Strömberg 2006). Modern phylogenetic comparative methods allow the distinction between convergent adaptations and similarities due to shared ancestry, and are thus an essential tool for the inference of adaptive evolution.

We revisit this classic story of adaptation in the light of new phylogenetic data (Fernández and Vrba 2005; Hassanin et al. 2012; Bibi 2013), improved comparative methods that allow for lagged adaptive evolution (reviewed in O'Meara 2012; Pennell and Harmon 2013; Hansen 2014; Mahler and Ingram 2014; O'Meara and Beaulieu 2014), and evidence for macroevolutionary lags in the emergence of both grasslands and hypsodonty itself (Strömberg 2006; Damuth and Janis 2011). We reconstruct time-calibrated phylogenetic relationships of Cetartiodactyla using existing molecular sequence data, and then reconstruct ancestral diet, habitat and geographic range on the inferred phylogenetic tree aided by fossil information on the emergence and spread of grasslands (reviewed in Strömberg 2011). We focus our analysis on ruminants since they represent the most diverse terrestrial suborder within Cetartiodactyla, are dominant herbivores in contemporary terrestrial open habitats, and cover a range of diets (Vrba and Schaller 2000).

To test whether diet or habitat played a major role in hypsodonty evolution in ruminants, we estimate both the degree of and temporal lag in adaptation toward different diet and habitat optima using the phylogenetic comparative framework initiated by Hansen (1997). In addition, we investigate if the appearance or the dominance of grasslands had the largest effect on the adaptation of hypsodonty toward these diet and habitat categories. This allows us to examine the temporal patterns of changes in hypsodonty in relation to the large-scale biotic shifts across continents on macroevolutionary scales. Lastly, we consider the evolution of ruminant body mass in relation to diet and habitat, since rumination has been shown to be advantageous over a limited range of body sizes (Demment and Van Soest 1985).

MATERIALS AND METHODS

Phylogenetic Relationships

In order to produce a robust timeline for the evolution of extant ruminants, we reanalyzed the molecular phylogenetic data set of Hassanin et al. (2012) with the software BEAST v.2.2.0 (Bouckaert et al. 2014), using two new sets of fossil calibrations that were recently presented by Bibi (2013) and Benton et al. (2015). The molecular data set of Hassanin et al. (2012) includes mitochondrial genomes of 184 nominal species of the order Cetartiodactyla and covers all extant families of this order as well as 81% and 55% of the currently described genera and species, respectively. In addition to 137 ruminant taxa, the data set of Hassanin

et al. (2012) includes 47 outgroup species within and outside of Cetartiodactyla, which allowed us to use four vetted fossil constraints on outgroup divergences following the recommendation of Benton et al. (2015) (see below in this paragraph). Including these outgroup species in our present study design also allowed us to infer the broader phylogenetic context of ruminants and to verify previously suggested clade interrelations within Cetartiodactyla (Hassanin et al. 2012) (Fig. 1). Phylogenetic reconstruction was therefore based on mitochondrial sequence data for each of the 184 species included in the data set of Hassanin et al. (2012). The molecular sequence data set of a total of 14,904 bp was split into five partitions according to transcript type (rRNA, 2085 bp; tRNA, 1461 bp; protein-coding mRNA, 7572 bp) and codon position (in the case of protein-coding genes). To account for potentially different evolutionary rates among these partitions, we used a separate model of sequence evolution for each of them. In separate sets of analyses, we applied either the HKY (Hasegawa et al. 1985) model, which allows independent rates for molecular transitions and transversions, or the GTR (Tavaré 1986) model of sequence evolution with six independent time-reversible substitution rates to all partitions. In both cases, we used a gamma distribution to model among-sites substitution rate variation within each of the five partitions. To time calibrate the ruminant part of the phylogeny, we employed a set of bovid fossil constraints that were identified and evaluated by Bibi (2013) by comparing divergence-time estimates obtained from analyses with single constraints and analyses with the full set of constraints. This set of constraints used in Bibi (2013) includes 16 fossil constraints, 1 of which was placed on the age of the most recent common ancestor of multiple individuals of *Alcelaphus buselaphus*. As we had limited our taxon set to a single individual for each of the 184 species (Supplementary Table S1 available on Dryad at <http://dx.doi.org/10.5061/dryad.b2v7v>), we had to exclude this constraint, but applied the other 15 bovid fossil constraints, with prior distributions as specified by Bibi (2013). In addition, we constrained the crown and stem ages of Cetacea, using lognormal divergence prior distributions with offsets according to fossil age and 95% quantiles as recommended by Benton et al. (2015). Furthermore, the earliest known member of Cetartiodactyla (*Diacodexis*) was used to constrain the stem age of this group, and the stem age of Carnivora was constrained with the age of *Ravenictis*, the oldest known member of this clade (Supplementary Table S1 available on Dryad). For both constraints, lognormal divergence prior distributions were applied, with 95% quantiles following Benton and Donoghue (2006). All clades used for fossil calibrations were constrained as monophyletic, and the sequence of divergences between Carnivora, Chiroptera, Perissodactyla, and Cetartiodactyla was constrained according to relationships found by Meredith et al. (2011) and O'Leary et al. (2013) based on genomic and large-scale morphological data sets, respectively. The

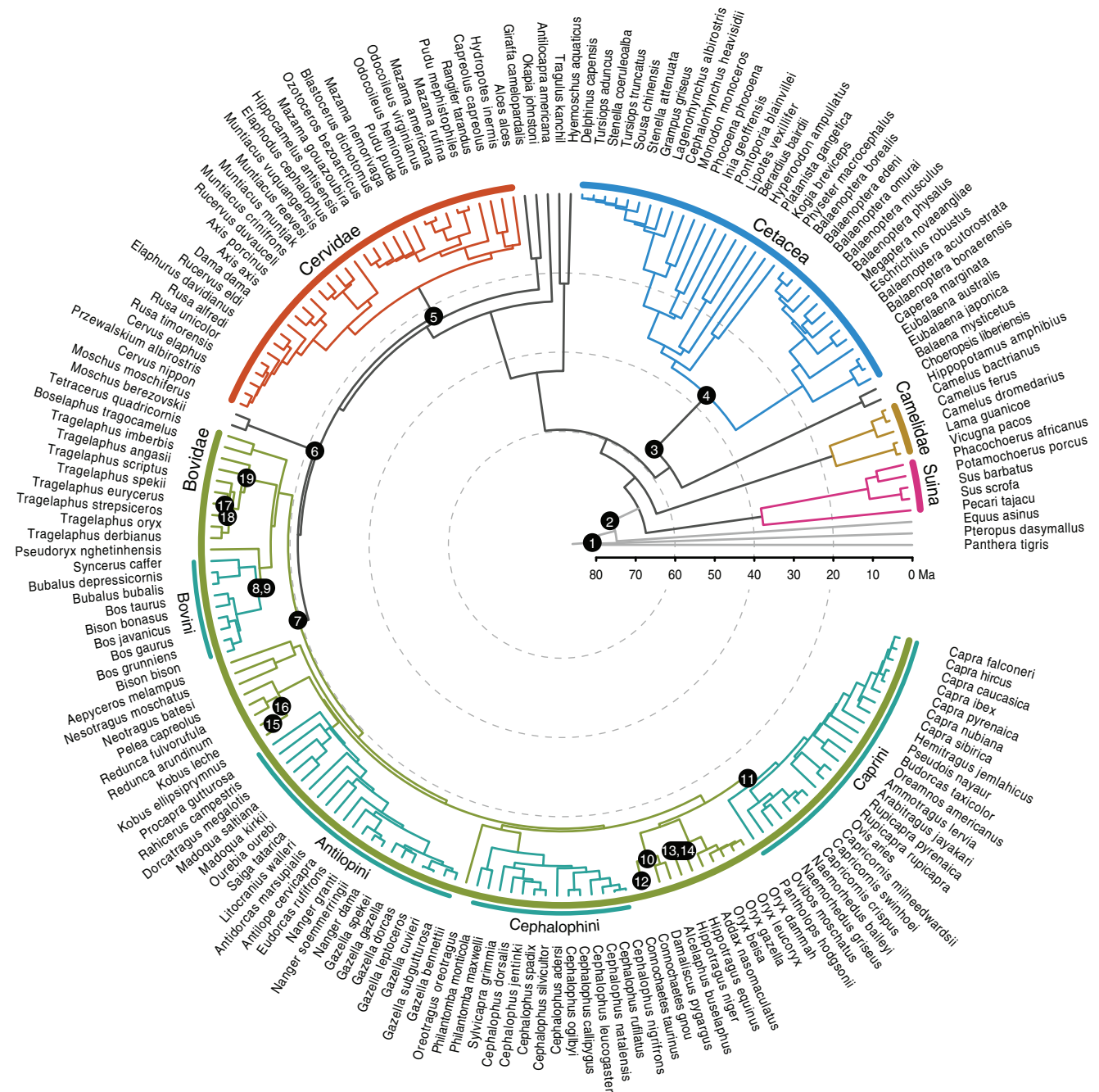


FIGURE 1. Phylogenetic tree of Cetartiodactyla. Branch lengths are proportional to time, the scale represents millions of years. All species are labeled, major lineages are color-coded and light-gray branches represent three noncetartiodactyl outgroup species. Numbered nodes (1–19) represent calibration points as given in Supplementary Table S1 available on Dryad.

reconstructed birth–death process (Gernhard 2008) was used as the tree prior, and branch rates were drawn independently from a lognormal distribution according to the uncorrelated lognormal clock model (Drummond et al. 2006).

For each of the two models of sequence evolution (HKY+ Γ and GTR+ Γ), we ran five replicate analyses with random starting trees, each for 100 million Markov chain Monte Carlo (MCMC) generations. Chain convergence was assessed based on effective

sample sizes greater than 200 for all parameters and by comparison of parameter traces within and among run replicates, using Tracer 1.6.0 (Rambaut et al. 2014). Parameter traces were also used for an a posteriori assessment of the relative fit of the HKY+ Γ and GTR+ Γ models, based on the Akaike information criterion through Markov chain Monte Carlo methods (AICM) (Raftery et al. 2007), calculated with BEAST v.1.8 (Drummond et al. 2012). After discarding the first 20% of posterior tree estimates from each run replicate as

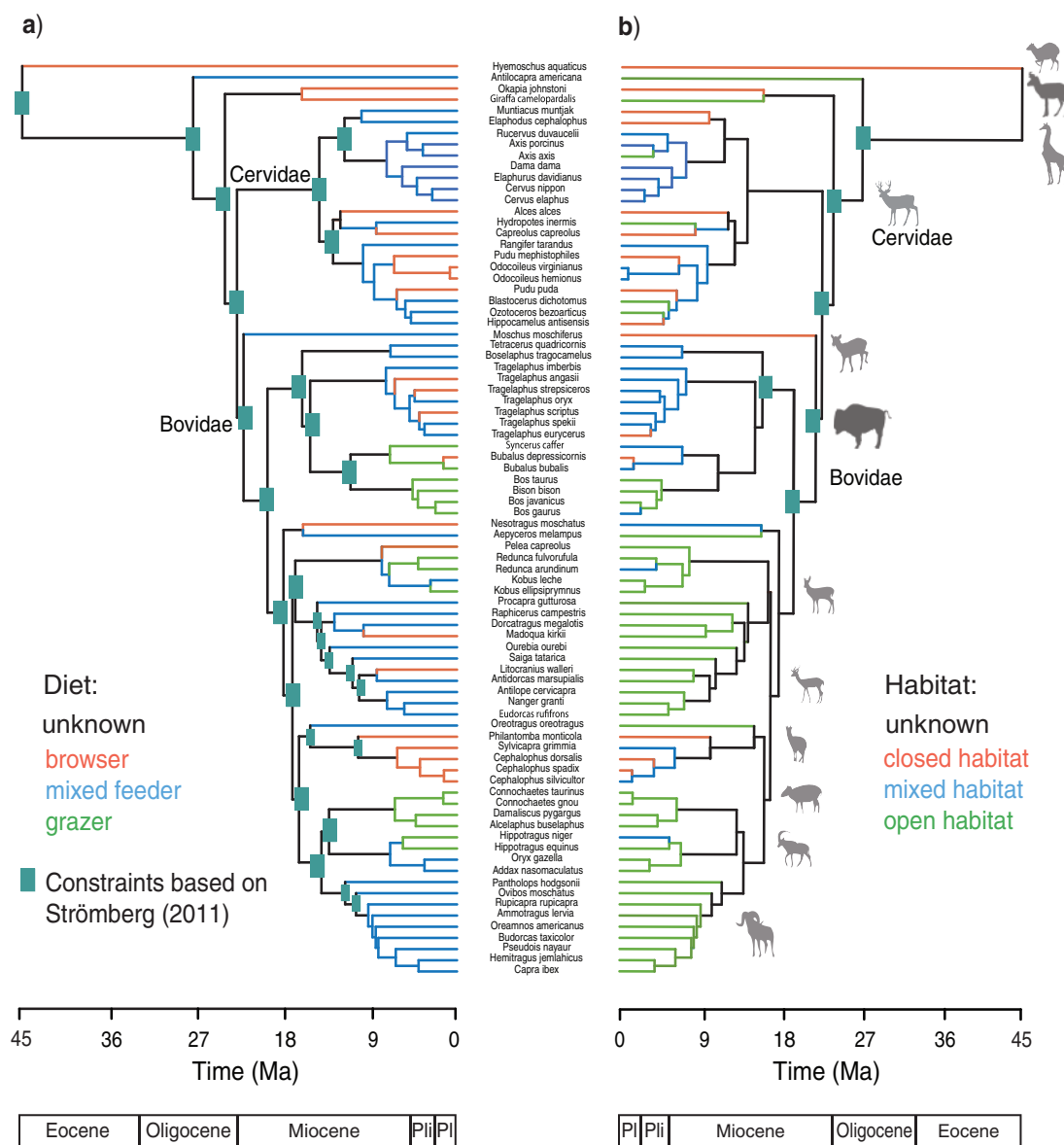


FIGURE 2. Ruminant phylogeny of 82 species with reconstructed diets and habitats. The two trees are mirror images with the tree in a) showing constrained ancestral reconstructions of dietary categories, and the tree in b) showing constrained ancestral reconstructions of habitat categories; positions of the teal rectangles mark the constraint of ancestral reconstructions based on Strömberg (2011). The branches painted black represent the corrections used for the best model (in bold) in Table 1. Illustrated major lineages include: Tragulidae, Antilocapridae, Giraffidae, Cervidae, Moschidae, Bovidae, Peleinae and Reduncinae, Antilopinae, *Oreotragus* and *Neotragus*, Cephalopinae, Alcelaphinae and Hippotraginae, Caprinae and Pantholopinae (top to bottom). Pl = Pleistocene; Pli = Pliocene.

burn-in, we combined the posterior tree distributions of replicates, thus producing a set of 10,000 posterior trees for each of the two models of sequence evolution. Maximum clade credibility trees were generated from these tree sets using TreeAnnotator v.2.2.0 (Bouckaert et al. 2014), with node heights according to the mean posterior clade age estimates.

Ancestral–Niche Reconstruction

As explained further below, our comparative analyses are based on mapping selective regimes (niche

categories) onto internal branches of a phylogeny as illustrated in Figure 2. Since phylogenetic uncertainty can bias ancestral-state reconstruction (Duchêne and Lanfear 2015), we reconstructed these regimes while considering phylogenetic uncertainty. To this end, we used an additional set of BEAST analyses to estimate the ancestral diet, habitat, and geographic range of internal nodes jointly with the time-calibrated species phylogeny. For this set of BEAST analyses, we used a GTR+ Γ model of sequence evolution, and settings as described above for BEAST analyses based on sequence data alone except that 150 million MCMC generations

per replicate were required for convergence. Ancestral niches were estimated, conditioned on the known current niches of the extant species in the phylogeny, using the probabilistic framework of Lemey et al. (2009). This framework was developed for the reconstruction of ancestral geographical distributions, but since it models these distributions as discrete traits, the framework can also be applied to any other set of discrete traits (Joy et al. 2016).

Diet and habitat data for 82 out of 137 (60%) extant ruminant species included in our tree were taken from Mendoza and Palmqvist (2008). Following these authors, we classified species into three diet and three habitat categories. Diet categories consisted of: (i) browsers, (ii) mixed feeders, and (iii) grazers. The categorization was made according to the percentage of grass in the species' diet (<25% for browsers, 25–75% for mixed feeders and >75% for grazers), based on the literature on diet of extant ungulates worldwide (Mendoza and Palmqvist 2008). Habitat categories were: (i) open habitat (i.e., treeless or scarcely wooded savannas, grasslands, dry deserts, and semidesert steppes); (ii) mixed habitat (i.e., wooded savannas, bush land, open forests, and species dwelling both in closed and open habitats); (iii) closed habitat (i.e., closed woodlands, riverine, moist deciduous, and evergreen forests). These were based on Mendoza et al. (2005). We lacked information on diet and habitat for 97 out of 184 extant species of Cetartiodactyla; those were estimated as a part of the BEAST analysis. Even though more data on ruminant diet are available in the literature (e.g., Cantalapiedra et al. 2014), these are not directly comparable to the categories we used. Geographic distributions (native occurrences) of extant taxa were assessed based on information from the IUCN Red List (2015) database, and were divided into six categories: (i) Africa, (ii) South America, (iii) North and Central America, (iv) Eurasia, (v) China and Southeast Asia, and (vi) marine. Marine taxa, outgroups, and ruminant taxa lacking data on hypsodonty were excluded from the comparative analyses, since our study focused on the evolution of hypsodonty.

Constraints of Ancestral–Niche Reconstruction Based on the Grassland Fossil Record

Ancestral-state reconstruction based exclusively on extant data may be misleading in cases where trait evolution has been directional over long periods of time (e.g., Finarelli and Flynn 2006). Thus we used the C₄ grass fossil record (summarized in Strömberg 2011) as independent information for the timing of appearance and dominance of different niches to constrain the reconstructed niches of internal nodes in our phylogenetic tree. The fossil information (Strömberg 2011) was used as an a posteriori constraint on ancestral-state reconstruction obtained from unconstrained models. Since recent evidence from grass fossils indicates lags between the appearance of grasses, their dominance in the ecosystem, and ungulates

utilizing these grasses (Edwards et al. 2010; Strömberg 2011), including grassland fossil constraints on the ruminant evolutionary timeline could prove valuable to understand the rate and timing of hypsodonty evolution. Besides, it would only be possible for species to be grazers or live in open habitats if grasslands were present on the continent they inhabited at the time. These constraints further allowed us to investigate and present a more fossil-based perspective of ruminant phenotypic adaptations in response to the grassland evolution. We focused on C₄ grasses since the fossil dating was both more abundant and more certain than that of C₃ grasses. Although the rise to dominance of C₄ grasslands through the replacement of C₃ grasslands was a complex event that is still being debated (Edwards et al. 2010), we assume that their global dominance in the present day grasslands justifies the use of their fossils as constraints. Additionally, the fact that the shift from C₃ to C₄ grasslands happened close to the root of our pruned phylogeny where very few lineages of ruminants are present would limit the potential impact of this event for the ruminant radiation and on our analysis.

We used both fossil dates of the first appearance of C₄ grasslands and those of dominance of C₄ grasslands for our constraints that were later the basis of our comparative approach. Since the dates for the first C₄ grasses are uncertain on some continents (~17 Ma for Africa, ~19 Ma for South Asia, and ~34 Ma for Western Eurasia), we could only constrain the assigned diet of six internal nodes and we made no changes to the habitat category. Seeing that the ages of the first appearance for C₄ grasslands are not the most reliable indicator that ruminants actually use C₄ grasses, we consider the niche reconstructions reflecting C₄ dominance (Fig. 1) to be a more biologically reliable indicator of ruminant adaptation to grasslands. These constraints are explained below.

Given that the minimum ages for grass fossils are well constrained, we assumed that lineages that lived on a continent before grasslands became dominant on that continent could not have had grazing as a part of their diet. Evidence from grass fossils (Strömberg 2011) suggests that C₄ grasslands became dominant in Africa and Central–South Asia around 9 Ma. In Eurasia, this happened earlier, about 11 Ma, whereas in South America the ages are more uncertain, yet point to a possible first appearance grasses as early as 40 Ma. Constraints on grassland evolution from North and Central America did not impact our analysis as no internal branches on the phylogeny were reconstructed as having lived on these continents. We assigned the reconstructed diet states of internal nodes conservatively to unknown if the mean node age predated the dominance of grasslands in the reconstructed geographical range (27 out of 163 nodes, see Fig. 2).

For habitat constraints, we took into account floral evidence from open grass-dominated habitats across continents, which suggest that no open habitats existed before 18 Ma in Africa and before 21 Ma in Eurasia

(Strömberg 2011). Estimates for other continents are more uncertain, but indicate that open habitats did not occur before 11 Ma and 40 Ma in Central and South Asia, and southern South America, respectively (Strömberg 2011). Thus, we constrained the reconstructed habitats of six internal nodes based on estimated node age and geographic range (Fig. 2) by changing their assignment from open habitat to unknown. Each time diet and habitat are considered in combination as a response variable, and one of these categories was marked as unknown, we used unknown to designate this species' niche.

Comparative Approach

We conducted phylogenetic comparative analysis using a model of adaptive evolution developed by Hansen (1997), as implemented in the SLOUCH software (Hansen et al. 2008; Labra et al. 2009; Escudero et al. 2012) in R, using the phylogeny in Figure 2.

This approach is based on modeling evolution as an Ornstein–Uhlenbeck process that can be represented by the stochastic differential equation:

$$dy = -\alpha(y - \theta(x))dt + \sigma dB,$$

in which dy is the change in trait y (hypsodontology index or body size in our case) over a time interval dt ; α is a parameter that measures how strongly the trait is attracted toward a central state, which we interpret as a “primary” optimum θ defined as an optimal state free of ancestral constraints (Hansen 1997); dB is a white-noise process (i.e., independent, normally distributed random values with mean zero and unit variance); and σ is the standard deviation of stochastic changes in the model.

The model can be used to study adaptation by allowing the primary optimum to take different values in different niches, indexed by x . The different niches in our analyses are determined by the predictor variables: diet, habitat, and log body mass. The diet and habitat niches are mapped on the phylogeny as explained above and different arrangements of niches corresponding to different historical hypotheses can be compared with Akaike information criteria (as in Butler and King 2004). The influence of (log) body mass on the primary optimum of hypsodont is modeled as a regression under the assumption that (log) body mass evolves as a Brownian motion (as in Hansen et al. 2008).

The α parameter is estimated as a phylogenetic half-life $t_{1/2} = \ln(2)/\alpha$, which can be interpreted as the average time it takes a species to move half the distance from an ancestral state toward a new optimum (Hansen 1997). A short half-life means rapid adaptation to a primary optimum while a long half-life implies that most species are adapting slowly and remain influenced by ancestral states for a long time. A half-life of $t_{1/2} = 0$ corresponds to instantaneous adaptation (no phylogenetic inertia), while a $t_{1/2} = \infty$ reduces the Ornstein–Uhlenbeck model to a Brownian motion process, although possibly with niche-dependent trends (see Hansen 1997). SLOUCH estimates the stochastic

component as a stationary variance $Vy = \frac{\sigma_y^2}{2\alpha}$, which is the predicted residual variance around the optima after the process has arrived at stochastic equilibrium. It can be interpreted as a measure of the influence of changes in unmeasured selective forces.

All models were tested with both the tree in which niche mapping accounted for the first appearance of C_4 grasslands and the tree in which niche reconstructions were based on dominance of C_4 grasslands (Fig. 2). Data on the hypsodonty index were taken from Janis (1988), in which it is defined as lower third-molar occlusal height divided by occlusal width. The sample size for the hypsodonty index per species reported in Janis (1988) ranges from one to four individuals, however only species means are reported. Data on mean body mass in kg were taken from Janis (1988), Pérez-Barbería and Gordon (2001), and Mendoza and Palmqvist (2008). Measures of uncertainty for the hypsodonty index and body mass were not available from these sources, and could therefore not be accounted for.

RESULTS

Phylogenetic Relationships

Phylogenetic analyses with different substitution models resulted in near-identical maximum clade credibility (MCC) tree topologies, differing only in the position of a single species, *Budorcas taxicolor*, which appeared as the sister of genera *Ammotragus* and *Arabitragus* in analyses based on the HKY model (posterior probability [PP] = 0.54), but closer to *Capra*, *Hemitragus*, and *Pseudois*, in trees based on the GTR model (PP = 0.59). Model comparison with the AICM (Raftery et al. 2007) provided strong support for the GTR model (AICM = 1,048,131; compared with AICM = 1,052,117 for the HKY model); thus all comparative analyses were based on the phylogeny estimated with the GTR model. Importantly, the tree topology was identical and divergence times differed by no more than 0.6 myr between analyses using molecular data alone and analyses including trait data for ancestral state reconstruction (Fig. 1). This means that the reconstruction of ancestral geography, diet, and habitat simultaneously with phylogenetic inference did not lead to circularity that could potentially result from inferring hypsodonty evolution on the basis of a phylogeny that was itself estimated with a data set that includes variables that may be related to hypsodonty.

Out of 183 nodes, only 21 received node support equal to or less than 0.99 in the MCC tree based on the GTR model. The topology of this tree is largely congruent with the maximum-likelihood phylogram of Hassanin et al. (2012), with the exception of seven topological differences: We recovered *Cephalorhynchus heavisidii* as the sister group of all other included members of Delphinidae (PP = 0.84), whereas Hassanin et al. (2012) found *Lagenorhynchus albirostris* in the same position, with low-bootstrap support (BS = 34%). Within Odocoileini, we found *Blastocerus dichotomus* to

TABLE 1. Parameter estimates for models of hypsodonty evolution including different predictor variables for niche reconstructions based on dominance of C₄ grasslands

Predictor variable	$\theta \pm \text{SE}$	$t_{1/2}$ (myr) (support region)	V_y	$R^2(\%)$	AICc
Single global optimum	2.38 ± 0.94	∞	$\sigma^2 = 2.62$	—	202.5
Diet	Unknown 3.11 ± 1.04	27.8 (13.4– ∞)	1.29	18.4	195.2
	Browser 0.07 ± 1.38				
	Mixed feeder 3.79 ± 1.54				
	Grazer 6.08 ± 2.47				
Habitat	Unknown 5.08 ± 2.49	15.7 (4.9– ∞)	0.87	24.6	193.3
	Closed habitat –0.11 ± 1.24				
	Mixed habitat 1.08 ± 1.57				
	Open habitat 3.03 ± 1.52				
Habitat × diet	Unknown 3.13 ± 0.83	11.6(10.8–45.2)	0.60	43.0	187.7
	Closed habitat browser 0.93 ± 0.62				
	Mixed habitat browser 2.26 ± 0.76				
	Open habitat browser 1.15 ± 0.82				
	Closed habitat mixed feeder 1.31 ± 0.85				
	Mixed habitat mixed feeder 2.30 ± 0.78				
	Open habitat mixed feeder 4.54 ± 0.65				
	Mixed habitat grazer 3.40 ± 1.22				
	Open habitat grazer 5.63 ± 1.06				
Log body mass	–35.45 ± 54.26	∞	$\sigma^2 = 2.60$	0.5	204.3

Note: Primary optima are given in the units of the trait (response variable) with standard error ($\theta \pm \text{SE}$). Half-life ($t_{1/2}$) values are in million years (myr) with two-unit support regions; stationary variance (V_y) is in units of trait squared; in models where $t_{1/2} = \infty$ we report σ^2 instead of variance V_y ; variance explained by the model (R^2) is shown in %; lower AICc scores indicate a better supported model. In bold: model with the shortest $t_{1/2}$ highest R^2 , and best AICc within the set of models presented in Table 1, which we chose as our overall best model. Primary optima (θ) estimates represent adaptive peaks in the given niche for hypsodonty index in Tables 1 and 2, and log body mass in Table 3.

form a clade with *Ozotoceros bezoarticus*, *Hippocamelus antisensis*, and *Mazama gouazoubira* (PP = 0.80), while the same species appears as the sister lineage to *Mazama nemorivaga* in the phylogeny of Hassanin et al. (2012) (BS = 16%). With strong support (PP = 1.0), we found the genus *Pseudoryx* as the sister of all other Bovini, even though the genus appears in a more nested position in the phylogeny of Hassanin et al. (2012) (BS = 38%). Within Reduncini, we recovered *Pelea* as the sister of a clade combining genera *Kobus* and *Redunca* (PP = 1.0), while it appears as the sister to *Kobus* alone in Hassanin et al. (2012) (BS = 69%). Tribe Reduncini appears as the sister of Antilopini (PP = 0.72) in our phylogeny, but is more nested in Antilopinae in Hassanin et al. (2012) (BS = 8%). The domestic sheep (*Ovis aries*) was found as the sister to genus *Rupicapra* in our phylogeny (PP = 0.62), but appears as the sister to all other members of Caprinae (BS = 48%) in Hassanin et al. (2012). Finally, our phylogeny supports a position of *Oreamnos americanus* as the sister to a clade combining genera *Budorcas*, *Pseudois*, *Capra*, and *Hemitragus* (PP = 0.76), but *Oreamnos* appears as the sister to only *Budorcas* in Hassanin et al. (2012) (BS = 48%).

Phylogenetic time calibration with 19 fossil constraints and lognormal divergence-time prior distributions suggests that Cetartiodactyla originated in the Campanian (mean = 74.8 Ma; 95% highest posterior density interval [HPD] = 80.6–69.1 Ma), and the most recent common ancestor of extant members of the order lived around the Cretaceous–Paleogene boundary (mean = 67.2 Ma; 95% HPD = 71.6–63.1 Ma). According to our phylogenetic age estimates, ruminants diverged from Whippomorpha, combining cetaceans and

hippopotamids, in the Paleocene (mean = 59.6 Ma; 95% HPD = 56.9–62.6 Ma), and crown ruminants originated in the Eocene (mean = 44.8 Ma; 95% HPD = 51.6–37.7 Ma). The major diversification of bovid lineages began in the Early Miocene (mean = 19.5 Ma; 95% HPD = 20.7–18.4 Ma), in agreement with age estimates from Hassanin et al. (2012), but slightly older than those obtained by Bibi (2013) for a phylogeny of Bovidae with a limited number of nonbovid outgroups (95% HPD = 17.3–15.1 Ma). The results from the BEAST inference of ancestral geographic ranges, as well as ancestral diet and habitat, are provided in the Supplementary Information available on Dryad.

Comparative Approach and the Evolution of Hypsodonty

Hypotheses based on either dominance (Table 1) or first appearance (Table 2) of C₄ grasslands gave qualitatively similar results and were supported almost equally by the AIC corrected for finite sample sizes (AICc) (Burnham and Anderson 2002), but we have higher confidence in the ancestral niche reconstructions based on dominance of C₄ grasslands (Table 1 and Fig. 2), as these are based on better supported ages of grassland fossils. Additionally, the opportunity for adaptation is likely to be much stronger for a dominant biome.

In the Ornstein–Uhlenbeck framework, a general phylogenetic effect (or signal) can be measured by the phylogenetic half-life estimated in a model that includes only a single global optimum (i.e., no predictor variables). For the hypsodonty index, this

TABLE 2. Parameter estimates for models of hypsodonty evolution including different predictor variables for niche reconstructions based on first appearance of C₄ grasslands (see Note below Table 1)

Predictor variable	$\theta \pm SE$	$t_{1/2}(\text{myr})$ (support region)	V_y	$R^2(\%)$	AICc
Single global optimum	2.38 ± 0.94	∞	$\sigma^2 = 2.62$	—	202.5
Diet	Unknown Browser Mixed feeder Grazer	26.4(10.8 – ∞)	1.26	18.5	195.4
Habitat	Closed habitat Mixed habitat Open habitat	22.8(8.1 – ∞)	1.10	21.2	191.0
Habitat × diet	Unknown Closed habitat browser Mixed habitat browser Open habitat browser Closed habitat mixed feeder Mixed habitat mixed feeder Open habitat mixed feeder Mixed habitat grazer Open habitat grazer	8.5(7.6 – 11.7)	0.50	50.4	184.1
Log body mass	−37.54 ± 53.77	∞	$\sigma^2 = 2.59$	0.6	204.2

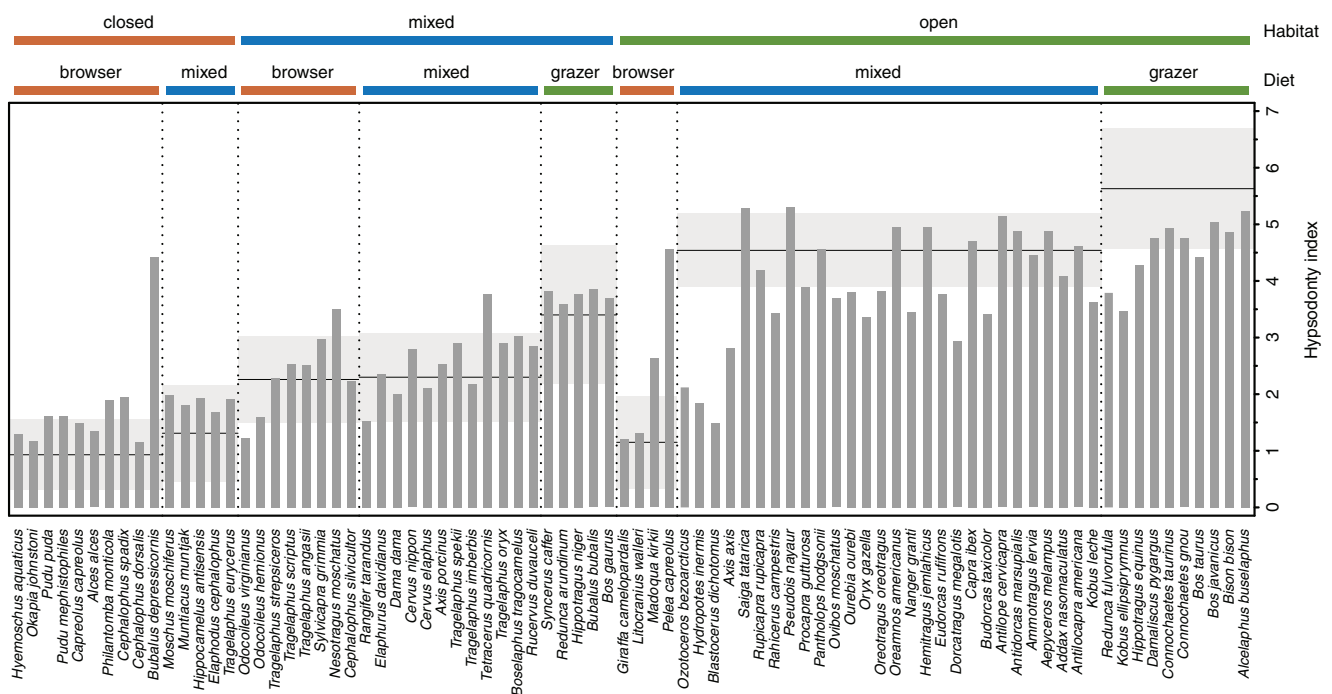


FIGURE 3. Data on hypsodonty index for extant ruminant species plotted with estimated primary optima from the best model (Table 1 in bold). Vertical gray bars represent the hypsodonty index (for right axis) of extant ruminant species. Dotted lines delimit different diet niches within habitats. Estimated primary optima for combinations of diet and habitat are represented by solid (horizontal) black lines, with gray areas around these lines representing 95% confidence intervals.

half-life estimate was infinity, which implies a strong phylogenetic signal indicating that hypsodonty evolves as if by Brownian motion. It is important to underscore that this is not a justification for the use of Brownian motion-based phylogenetic generalized least squares or independent-contrasts analyses, because these methods require that the residuals from the analysis follow a Brownian motion process, and not the species trait values themselves (Labra et al. 2009; Revell 2010; Hansen 2014).

The model including both diet and habitat as predictors (Table 1) had the best AICc value and explained around 43% of the variance in the hypsodonty index when niche reconstructions were based on dominance of C₄ grasslands. Diet and habitat had roughly equal and additive effects on the hypsodonty optima, explaining in isolation around 18% and 25% of the trait variance, respectively. The estimated optima (Table 1) confirm hypsodonty as an adaptation to both

TABLE 3. Parameter estimates for models of body mass evolution including different predictor variables for niche reconstructions based on first dominance of C₄ grasslands (see Note below Table 1)

Predictor variable	$\theta \pm SE$	$t_{1/2}$ (myr) (support region)	V_y	$R^2(\%)$	AICc
Single global optimum	1.57 \pm 0.56	∞	$\sigma^2 = 0.92$	—	116.8
Diet	Unknown 1.77 \pm 0.56	16.6(7.6 – ∞)	0.33	12.3	111.7
	Browser 1.34 \pm 0.50				
	Mixed feeder 1.67 \pm 0.55				
	Grazer 3.84 \pm 0.91				
Habitat	Unknown 1.04 \pm 1.43	24.2(9.0 – ∞)	0.45	6.62	116.3
	Closed habitat 1.37 \pm 1.22				
	Mixed habitat 2.98 \pm 1.53				
	Open habitat 2.87 \pm 1.48				
Habitat \times diet	Unknown 1.71 \pm 0.54	13.9(5.8 – 132.96)	0.27	21.3	116.1
	Closed habitat browser 1.07 \pm 0.46				
	Mixed habitat browser 1.12 \pm 0.67				
	Open habitat browser 2.44 \pm 0.61				
	Closed habitat mixed feeder 1.07 \pm 0.63				
	Mixed habitat mixed feeder 2.18 \pm 0.59				
	Open habitat mixed feeder 1.75 \pm 0.49				
	Mixed habitat grazer 4.19 \pm 0.92				
	Open habitat grazer 3.52 \pm 0.80				

grazing and open habitats, with open-habitat grazers having an estimated primary optimum of 5.6 for the hypsodonty index. As can be seen in Figure 3, all extant grazers are considerably less hypsodont than their estimated primary optimum. Considering the estimated phylogenetic half-life of about 12 myr, our interpretation is that they have not had enough time to adapt fully to grasslands. In contrast, closed-habitat browsers tend to be more hypsodont than their estimated primary optimum, which may be due to some extant species in this category having evolved from presumably more hypsodont ancestors in other niches. The estimated optimum of open-habitat browsers (Table 1 and Fig. 3) comes with a caveat, as there are only four species in this category in our data set. Caution is also needed when interpreting the estimated optima for two other categories with only five species (closed-habitat mixed feeders and mixed-habitat grazers). Phylogenetic half-lives tend to become shorter when more predictors are added. Diet and habitat alone gave half-lives of about 28 and 16 myr, respectively. This is an indication that lags in a focal adaptation may be due to adaptation to unmeasured niche variables not explicitly included in the models.

We obtained qualitatively similar results when ancestral niche reconstructions were based on first appearance of C₄ grasslands (Table 2). Here also, diet and habitat had roughly equal and additive effects on hypsodonty optima (explaining around 18% and 21% of the variance, respectively). The best-supported model was again the one that included the combination of diet and habitat, with slightly lower optima estimates compared with the results based on dominance of C₄ grasslands. This is a result of the shorter estimated half-life. The hypothesis based on the appearance of the C₄ grasslands included only 6 nodes in the unknown category, while the hypothesis based on dominance of C₄ grasslands included 27 nodes mapped as unknown.

Body mass had no effect on the hypsodonty optima (Tables 1 and 2). When included as a single predictor variable, log body mass explained less than 1% of the variance. When considered together with other variables, it did not lead to a significantly better fit of the model (Supplementary Table S2 available on Dryad).

Evolution of Body Mass

Body mass in isolation also showed a pattern of evolution with a strong phylogenetic signal, resembling Brownian motion with a half-life of infinity (Table 3). In contrast to their effects on hypsodonty, diet and habitat did not seem to be important for body-mass evolution, jointly explaining only 21% of the variance (Table 3). The best model according to AICc included diet alone and explained only about 12% of the variance. The estimated primary optima for grazers, compared with browsers and mixed feeders, indicate a tendency for larger body mass in grazers. Nonetheless, the low variance explained, coupled with the long half-life of around 17 myr shows the sparse predictive value of diet and even more so of habitat for ruminant body-mass evolution.

DISCUSSION

Hypsodonty evolution has mainly been discussed without the aid of rigorous quantitative analyses including information on the evolution of grasslands. Recent debates on the importance of diet and habitat for hypsodonty evolution (Strömberg 2006; Jardine et al. 2012; see Damuth and Janis 2011 for review) have made the need for quantitative tests more evident (Williams and Kay 2001; Muhlbachler and Solounias 2006). It has also been suggested that there is an apparent adaptive lag between the appearance of grasslands and the evolution of highly hypsodont forms in the

fossil record (Hansen 1997; Muhlbachler and Solounias 2006; Strömberg 2006; Muhlbachler et al. 2011). Our comparative analysis, informed by the fossil record of grassland spread across different continents, shows an independent and almost equal contribution of diet and habitat to hypsodonty evolution. Consequently, their combined effect is the best predictor of hypsodonty in present-day ecosystems. Additionally, the negligible effect of body mass on hypsodonty evolution lends support to suggestions that hypsodonty is independent of body size in ruminants (Fortelius 1985; Janis 1988). Moreover, we found only minor effects of diet and habitat on the evolution of body size in ruminants.

Only a handful of studies have looked at the contribution of different factors to adaptive explanations of hypsodonty in a phylogenetic framework (Hansen 1997; Williams and Kay 2001; Muhlbachler and Solounias 2006). These studies found contrasting results on the importance of diet and habitat, which could be a result of differences in model assumptions, data used to quantify tooth wear, taxa analyzed, and/or the accuracy of the analyzed phylogenies. Williams and Kay (2001) found the impact of diet and foraging height most important in ungulates, and no impact of habitat. Muhlbachler and Solounias (2006) measured mesowear (Fortelius and Solounias 2000) and found no coevolutionary correlation of diet and crown height in oreodonts. Both studies used Felsenstein's (1985) independent-contrasts method. Hansen (1997) used the same adaptation model as in our analysis, and found a strong, though lagged effect of diet on hypsodonty in horses. This approach does not only have the advantage of estimating and correcting for lag in adaptation due to phylogenetic inertia but also allow for a more accurate phylogenetic correction based on residual correlations, as it is strictly these and not general phylogenetic effects that should be statistically corrected for in using generalized least squares.

With more appropriate methods, and a substantially larger data set for the phylogenetic relationship and the reconstructed ancestral niches we were able to test different hypotheses of hypsodonty evolution in a more rigorous way. Our time-calibrated phylogenetic tree is based on a number of recently described fossil calibrations, both within Ruminantia (Bibi 2013) and early in the tree of Cetartiodactyla (Benton et al. 2015), and is well resolved and highly supported. Using fossil data to support ancestral niche reconstructions is also crucial to constrain the time between the first appearance of grassland and their rise as the dominant ecosystem and a major factor in ungulate diets (Strömberg 2011), which is essential to disentangle the complex story of hypsodonty evolution. Although dietary transitions and diversification in ruminants have previously been compared with fossil-based timing of grassland expansion (e.g., Cantalapiedra et al. 2014), we did this more directly by including the timeline of grasslands evolution in the sets of models of hypsodonty evolution by comparing models fitted to ancestral niche arrangements that were corrected for either first

appearance or dominance of C₄ grasslands. The dates for the first appearance of C₄ are highly uncertain and near the root of the ruminant tree. For these reasons we consider niche arrangements based on the dominance of C₄ open grasslands to be more reliable and relevant for hypsodonty evolution. However, models based on first appearance and dominance of C₄ grasslands gave qualitatively similar results.

Recently Cantalapiedra et al. (2014) reconstructed dietary shifts in ruminants and related these to rates of diversification and the timing of grassland evolution. They concluded that grazing evolved through a period of mixed feeding, although with highly reversible transitions between diets, not following a simple unidirectional model. The results show that mixed feeding and grazing largely predates the late Miocene transition to C₄ grasslands. Although our niche mapping is consistent with grazing arising from mixed feeding, widespread grazing before the late Miocene would expand the grazing niches on our phylogeny (Fig. 2a), and this would most likely reinforce our main findings by producing even longer lags and more pronounced maladaptation in hypsodonty. Ancestral states reconstructed from models based on extant data can be unreliable, however, and we feel more confident with niche reconstructions constrained by fossil information.

Slow adaptation is not uncommon on macroevolutionary time scales (Labandeira 2007; Uyeda et al. 2011; Hansen 2012; Voje and Hansen 2013; Voje et al. 2014). Long half-lives of around 10 myr suggest slow evolution of hypsodonty toward estimated optimal states. Given the uncertainties in the estimated hypsodonty index for each species because of low sample sizes, and the broad niches we mapped on the phylogeny, the finding that close to half the interspecies variance can be explained by diet and habitat is remarkable, as well as a good indication of their importance for hypsodonty evolution. Assuming that most of the unexplained variance is due to adaptation to unobserved niche variables, we expect that phylogenetic half-lives would be shorter if a more refined reconstruction of the niches would be included. The estimated half-lives are in fact substantially shorter when both habitat and diet are included as predictors compared with models with only one or the other. Regardless, some of the unexplained variation in hypsodonty may be because of adaptation toward optima influenced by climate variables (e.g., aridity) and morphological characters that high-crowned teeth depend on (e.g., teeth and skull traits). Nevertheless, existence of a time lag in trait adaptation should be taken into account when hypsodonty is used as a proxy for different paleoenvironments and climates (Strömberg 2006). Present-day grazing lineages such as wildebeest and hartebeest seem not to have reached their estimated optimal tooth height; therefore a potential time lag needs to be addressed when fossils are used to infer diets and habitats. This is particularly important because the lag is most apparent in the highly hypsodont grazing forms

that are often used as proxies, whereas taxa from other categories are generally closer to their estimated optima (Fig. 3).

Initially absent or weak selection combined with periodic increases in selection intensity (Strömberg 2006; Mühlpachler et al. 2011) has been suggested as an explanation for the adaptive lag in hypsodont horses in relation to grassland evolution. This is not plausible from a population-genetics perspective, however; very weak selection, or extremely short periods with intense selection would be sufficient to produce instantaneous adaptation and extreme hypsodonty on the time scale in question, while the absence of selection would lead to much larger changes than observed (Lynch 1990; Hansen 2012). The causes of the macroevolutionary lag are therefore more likely to be found in some form of genetic or ecological constraint. Behavioral plasticity—avoiding grass consumption as long as alternative food sources such as browse are available—has also been suggested as another potential explanation of what could have moderated the selection pressures (Strömberg 2006).

Although we have a good representation of all ruminant tribes, which should provide a fairly unbiased subset of niches, the diet and habitat categories used in our analysis represent a very broad division of such niches. Nevertheless, there are some discrepancies between diet and habitat classifications of some species in our data set and the literature. The pronghorn (*Antilocapra americana*) and the takin (*Budorcas taxicolor*) were both designated as mixed feeders in our data set, whereas Fortelius and Solounias (2000) designated these species as strict browsers. Similarly, the lowland anoa (*Bubalus depressicornis*) was marked as a browser in our data set as well as in Burton et al. (2005), but as a mixed feeder in Flores-Miyamoto et al. (2005). In order to keep category assignments consistent between species, we exclusively used the data set of Mendoza and Palmqvist (2008) for diet, which provided the largest overlap in species coverage with our phylogenetic tree and with the hypsodonty data. We tested for the effect of these disagreements on our analyses by changing the diet of several recent species according to the differences reported in the literature (Supplementary Table S2 available on Dryad). Results obtained with these models were qualitatively similar to those based on our best-fitting model, with longer estimates of half-lives and less variance explained (Supplementary Table S2 available on Dryad). Previous studies (Janis 1988; Pérez-Barbería and Gordon 2001; Williams and Kay 2001; Mendoza et al. 2002; Mendoza and Palmqvist 2008) have provided strong support for grit as a driver of the evolution of hypsodonty, whereas in our study, diet was about as important as habitat, which may be taken as a proxy for levels of grit. This could be because of the broad habitat categories used in our analyses, or varying definitions of these categories in the different studies. The habitat categories used here do not fully account for the external grit, which has been proposed

as an important and sometimes overlooked factor in the evolution of hypsodont teeth (Janis 1988; Damuth and Janis 2011). A way to include grit in the analysis would be to look at the height of the vegetation at which the animals are feeding, and adjust diet and habitat categories accordingly (see Janis 1988; Williams and Kay 2001).

Apart from improving estimates of the tested model parameters, an obvious next step would be to incorporate climate variables, such as precipitation, in models of ruminant tooth evolution. Previous studies have found a strong relationship between local mean hypsodonty and local mean annual precipitation in both modern (Fortelius et al. 2002) and paleo-environments (Eronen et al. 2010a; 2010b). Additionally, more data on paleo-environments, such as more grass fossil data with better temporal resolution would be required to directly include these fossils into the ancestral reconstructions, rather than in correcting contradicting nodes a posteriori. Isotopic data on grass fossils, as well as teeth, can provide us with a better understanding of the evolutionary patterns in the fossil record (Uno et al. 2011; Cantalapiedra et al. 2014; Cerling et al. 2015). Including measures of unworn teeth from extinct taxa would be invaluable, but completely unworn molars are rarely found in fossils. Furthermore, a single measure such as hypsodonty is an incomplete measure of dietary adaptation, and it would be beneficial to take into account more measures such as postcanine tooth volume, 3D tooth measurements, mesowear, and microwear (Janis 1988, 1995; Fortelius and Solounias 2000; Cantalapiedra et al. 2014). Lastly, future analyses should include measurement error stemming from estimation error in the species statistics used as variables in the comparative analysis (e.g., Hansen and Bartoszek 2012; Garamszegi 2014).

Evolution of ruminant hypsodonty is a complex story and is therefore vital to include the information from the grasslands' fossil record in order to elucidate the tempo and mode of ruminant adaptations. The combined effects of diet and habitat seem to have played a substantial role in hypsodonty evolution, and future studies should focus on examining their joint influence rather than their individual contributions. Answers to why adaptation of hypsodonty proceeded at a slow pace might potentially lie in the variance that has not been accounted for, as well as in genetic, morphological, developmental or ecological constraints. Nevertheless, our results, together with the previously recovered time discordance between the grasslands and hypsodonty evolution, point to a slow evolution of hypsodonty.

SUPPLEMENTARY MATERIAL

Data available from the Dryad Digital Repository: <http://datadryad.org>, <http://dx.doi.org/10.5061/dryad.b2v7v> and TreeBASE repository of phylogenetic

information at <https://treebase.org>, <http://purl.org/phylo/treebase/phyloids/study/TB2:S21191>.

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